



Appl. No. 09/836,705
Reply to Office Action dated May 29, 2003

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claim 1. (withdrawn) A polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a protein having the amino acid sequence of SEQ ID NO 38, or a polynucleotide variant thereof encoding a modified amino acid sequence having at least one deletion, addition, substitution or alteration, said polynucleotide variant being capable of accelerating the biosynthesis of ML-236B; and

(b) a polynucleotide encoding a protein having the amino acid sequence of SEQ ID NO 42, or a polynucleotide variant thereof encoding a modified amino acid having at least one deletion, substitution or alteration, said polynucleotide variant being capable of accelerating the biosynthesis of ML-236B.

Claim 2. (withdrawn) A polynucleotide according to claim 1 comprising a mutant or variant of SEQ ID NO 37 capable of accelerating the biosynthesis of ML-236B.

Claim 3. (withdrawn) A polynucleotide according to claim 1, comprising SEQ ID NO. 37.

Claim 4. (withdrawn) A polynucleotide according to claim 1 comprising DNA obtained from transformed *Escherichia coli* pSAKexpE SANK 72499 (FERM BP-7005).

Claim 5. (withdrawn) A polynucleotide according to claim 1 comprising a variant of SEQ ID NO 41 capable of accelerating the biosynthesis of ML-236B.

Claim 6. (withdrawn) A polynucleotide according to claim 1, comprising SEQ ID NO 41.

Claim 7. (withdrawn) A polynucleotide according to claim 1 comprising DNA obtained from transformed *Escherichia coli* pSAKexpR SANK 72599 (FERM BP-7006).

Claim 8. (withdrawn) A polynucleotide according to claim 1 in combination with one or more other polynucleotides, said combination being capable of enhancing the production of ML-236B in a ML-236B producing micro-organism.

Claim 9. (withdrawn) A polynucleotide according to claim 3 in combination with one or more other polynucleotides, said combination being capable of enhancing the production of ML-236B in a ML-236B producing micro-organism.

Claim 10. (withdrawn) A polynucleotide according to claim 4 in combination with one or more other polynucleotides, said combination being capable of enhancing the production of ML-236B in a ML-236B producing micro-organism.

Claim 11. (withdrawn) A polynucleotide according to claim 6 in combination with one or more other polynucleotides, said combination being capable of enhancing the production of ML-236B in a ML-236B producing micro-organism.

Claim 12. (withdrawn) A polynucleotide according to claim 7 in combination with one or more other polynucleotides, said combination being capable of enhancing the production of ML-236B in a ML-236B producing micro-organism.

Claim 13. (withdrawn) A polynucleotide according to claim 8 comprising a polynucleotide of SEQ ID NO 37, or a variant thereof, in combination with one or more sequences selected from the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 14. (withdrawn) A polynucleotide according to claim 9 comprising a polynucleotide of SEQ ID NO 37, or a variant thereof, in combination with one or more sequences selected from

the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 15. (withdrawn) A polynucleotide according to claim 10 comprising a polynucleotide of SEQ ID NO 37, or a variant thereof, in combination with one or more sequences selected from the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 16. (withdrawn) A polynucleotide according to claim 11 comprising a polynucleotide of SEQ ID NO 37, or a variant thereof, in combination with one or more sequences selected from the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 17. (withdrawn) A polynucleotide according to claim 12 comprising a polynucleotide of SEQ ID NO 37, or a variant thereof, in combination with one or more sequences selected from the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 18. (withdrawn) A polynucleotide according to claim 8 comprising a polynucleotide of SEQ ID NO 41, or a variant thereof, in combination with one or more sequences selected from the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 19. (withdrawn) A polynucleotide according to claim 9 comprising a polynucleotide of SEQ ID NO 41, or a variant thereof, in combination with one or more sequences selected from the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 20. (withdrawn) A polynucleotide according to claim 10 comprising a polynucleotide of SEQ ID NO 41, or a variant thereof, in combination with one or more sequences selected from the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 21. (withdrawn) A polynucleotide according to claim 11 comprising a polynucleotide of SEQ ID NO 41, or a variant

thereof, in combination with one or more sequences selected from the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 22. (withdrawn) A polynucleotide according to claim 12 comprising a polynucleotide of SEQ ID NO 41, or a variant thereof, in combination with one or more sequences selected from the group consisting of SEQ ID NO 37, SEQ ID NO 41, SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47, SEQ ID NO 49 and variants thereof.

Claim 23. (withdrawn) A polynucleotide capable of hybridizing under stringent conditions with a polynucleotide according to claim 1.

Claim 24. (withdrawn) A polynucleotide according to claim 23 capable of accelerating the biosynthesis of ML-236B in a ML-236B producing micro-organism when introduced in the ML-236B producing micro-organism.

Claim 25. (withdrawn) A polynucleotide according to claim 23 or 24 which is RNA.

Claim 26. (withdrawn) A vector comprising a polynucleotide according to claim 1.

Claim 27. (withdrawn) A vector according to claim 26 obtained from *Escherichia coli* pSAKexpE SANK 72499 (FERM BP-7005) or *Escherichia coli* pSAKexpR SANK 72599 (FERM BP-7006).

Claim 28. (withdrawn) A vector according to claim 26 or 27 which is an expression vector.

Claim 29. (withdrawn) A host cell transformed by a vector according to claim 26 or 27.

Claim 30. (withdrawn) A host cell according to claim 29, wherein the host cell is a ML-236B producing micro-organism.

Claim 31. (withdrawn) A host cell according to claim 30, wherein the host cell is *Penicilium citrinum*.

Claim 32. (withdrawn) A host cell according to claim 29, wherein the host cell is *Escherichia coli*.

Claim 33. (withdrawn) A host cell according to claim 32, wherein the host cell is *Escherichia coli* pSAKexpE SANK 72499 (FERM BP-7005).

Claim 34. (withdrawn) A host cell according to claim 32, wherein the host cell is *Escherichia coli* pSAKexpR SANK 72599 (FERM BP-7006).

Claim 35. (withdrawn) A polypeptide encoded by a polynucleotide according to claim 1.

Claim 36. (withdrawn) A polypeptide comprising the sequence of SEQ ID NO 38, or a variant thereof which has at least 80%

identity to SEQ ID NO 38 and which is capable of accelerating ML236B production in an ML236B producing organism.

Claim 37. (withdrawn) A polypeptide according to claim 36, having the sequence of SEQ ID NO 38.

Claim 38. (withdrawn) A polypeptide comprising the sequence of SEQ ID NO 42, or a variant thereof which has at least 80% identity with SEQ ID NO 42 and which is capable of accelerating ML236B production in an ML236B producing organism.

Claim 39. (withdrawn) A polypeptide according to claim 38, having the sequence of SEQ ID NO 42.

Claim 40. (currently amended) A method for producing ML-236B[[,]] comprising

(a) culturing a host cell ~~according to claim 29~~ transformed by a vector comprising a polynucleotide encoding a protein having the amino acid sequence of SEQ ID NO 42, or a polynucleotide variant thereof encoding a modified amino acid sequence having at least one deletion, substitution or alteration, said

polynucleotide variant being capable of increasing the transcription of a gene selected from the group consisting of mlcA, mlcB, mlcC and mlcD, said polynucleotide variant having at least an 80% identity with SEQ ID NO 42, and then

(b) recovering ML-236B from the resultant culture.

Claim 41. (currently amended) A method according to claim 40, wherein the host cell is transformed with a vector comprising ~~SEQ ID NO 37~~ or a polynucleotide having the nucleotide sequence of SEQ ID NO 41.

Claim 42. (currently amended) A method according to claim 41, wherein the vector comprises no additional genes which comprise a polynucleotide having the nucleotide sequence of any one of SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47 or SEQ ID NO 49.

Claim 43. (currently amended) A method according to claim 40, wherein the producing of ML-236B occurs in the absence of a recombinant gene encoding mlcA corresponding to SEQ ID NO 44, a recombinant gene encoding mlcB corresponding to SEQ ID NO 46, a

recombinant gene encoding mlcC corresponding to SEQ ID NO 48 or a
recombinant gene encoding mlcD corresponding to SEQ ID NO 50.

Claim 44. (withdrawn) ML-236B produced by the method of claim 40.

Claim 45. (currently amended) A method of manufacturing
pravastatin, which comprises carrying out ~~a~~ the method according
to claim 40 and converting the ML-236B to pravastatin.

Claim 46. (withdrawn) An antibody reactive with the protein of
SEQ ID NO 38 or SEQ ID NO 42.

Claim 47. (withdrawn) A polynucleotide encoding a protein having
the amino acid sequence selected from the group consisting of SEQ
ID NO 44, SEQ ID NO 46, SEQ ID NO 48 and SEQ ID NO 50, or a
variant polynucleotide encoding a modification of said amino acid
sequence having a deletion, substitution, addition or
alteration, said variant polynucleotide being capable of
accelerating the biosynthesis of ML-236B.

Claim 48. (withdrawn) A polynucleotide according to claim 47 selected from the group consisting of SEQ ID NO 43, SEQ ID NO 45, SEQ ID NO 47 and SEQ ID NO 49.

Claim 49. (withdrawn) A polynucleotide according to claim 47 or 48, said polynucleotide being capable of accelerating the biosynthesis of ML-236B alone or in conjunction with the polynucleotide of SEQ ID NO 37 or SEQ ID NO 41.

Claim 50. (withdrawn) A vector comprising a polynucleotide according to claim 47 or 48.

Claim 51. (withdrawn) A host cell comprising a vector according to claim 50.

Claim 52. (withdrawn) A polypeptide encoded by a polynucleotide according to claim 47 or 48.

Claim 53. (withdrawn) A method for the production of ML236B comprising culturing a host cell according to claim 51 and then recovering ML-236B from the culture.

Claim 54. (withdrawn) A polynucleotide according to claim 1, wherein the polynucleotide encodes a protein having the amino acid sequence of SEQ ID NO. 38.

Claim 55. (withdrawn) A polynucleotide according to claim 1, wherein the polynucleotide encodes a protein having the amino acid sequence of SEQ ID NO 42.

Claim 56. (new) A method for producing ML-236B comprising
(a) culturing a host cell transformed by a vector which is pSAKexpR that is obtained from *Escherichia coli* pSAKexpR SANK 72599 (FERM BP-7006) and
(b) recovering ML-236B from the resultant culture.

Claim 57. (new) A method of manufacturing pravastatin comprising carrying out the method according to claim 56, and converting the ML-236B to pravastatin.

Claim 58. (new) A method according to claim 40, wherein the vector is pSAKexpR which is obtained from *Escherichia coli* pSAKexpR SANK 72599 (FERM BP-7006).

Claim 59. (new) A method according to claim 40, wherein the polynucleotide consists of SEQ ID NO 41.

Claim 60. (new) A method according to claim 40, wherein the polynucleotide encodes a protein consisting of the amino acid sequence of SEQ ID NO 42.